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TOXICOLOGICAL STUDIES OF SMOKE OBSCURANTS

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ABSTRACT

An exposure facility was designed and constructed to support health effect studies of inhaled smoke obscurants generated from light lubricating oils. The exposure system is capable of providing smoke atmospheres in the concentration range of 0.4 - 10 mg oil/liter chamber air with an aerosol size of approximately 0.5 μ m mass median aerodynamic diameter (MMAD), and a geometric standard deviation (σ) of approximately 1.5. Concentrations are monitored using gravimetric filter sample analysis and continuous RAM-1 aerosol monitors. Chemical consistency of the test atmospheres is monitored by observing the relative stability of fractions of the oil as measured by high performance liquid chromatographic analysis of collected aerosol samples. Sixty-day-old Fischer 344 and Sprague Dawley rats were used in a series of exposures conducted at 0.5 and 1.5 mg oil/liter chamber air over a four week period at 70 minutes/day and 3.5 hours/day for 2 days/week or 4 days/week. Toxicological parameters investigated include: clinical chemistry (i.e. bilirubin, cholinesterase, LDH, SGOT, and SGPT), and hematology; immunology (i.e. natural killer cell activity and lymphocyte response to mitogens); protein content of pulmonary lavage fluid; pulmonary free cells (i.e. viability, phagocytic rates, and differential counts); pathology; cardiopulmonary physiology on unanesthetized animals studying dynamic responses such as lung compliance, airway resistance, electrocardiograms and blood gases; pulmonary physiology of anesthetized rats including lung volumes, diffusion capacity of CO, and nitrogen washout; xenobiotic metabolism (i.e. pentobarbital-induced sleeping time and zoxazolamine-induced paralysis time). This work is supported by the U.S. Environmental Protection Agency under Contract #68-02-4032.

1. INTRODUCTION

Fog oil, the smoke obscurant generated by vaporization and subsequent condensation of light petroleum oils, is one of many chemical smokes and obscurants used by the military to conceal personnel, material, and installations from visual observation. Military personnel may be exposed to fog oil for hours within a single day, and exposures may be repeated over consecutive days. Evaluation

of the potential hazards posed by fog oil to human health is a necessary portion of the data base required to establish comprehensive health criteria for the field use of smokes and obscurants. To evaluate these potential health effects, research was initiated with the following objectives:

- 1) to design and develop an inhalation exposure facility which could be used to evaluate the biological effects of fog oil in experimental animals, 2) to apply, with any needed modifications, methods for determining the physical and chemical characteristics of the smoke aerosol present in the exposure chambers, 3) to make toxicological evaluation in rats exposed to the fog oil by estimating lethality (LC_{50}) for several exposure durations, and evaluating histopathology in nasal, tracheobronchial, and alveolar regions of animals exposed to lethal doses, 4) to determine in short-term (4 week) studies biologic endpoints, durations of exposure, frequencies of exposure, and concentrations of interest for subchronic (90 day) studies, and 5) to conduct studies on effects of subchronic (90 day) exposures. Progress toward these objectives is presented below.

2. EXPOSURE FACILITY DESCRIPTION

To perform studies on the health effects of inhaled fog oil aerosol, a facility for animal inhalation exposures and biological testing of exposed animals was designed, constructed, and tested.

The fog oil exposure facility consists of an exposure control laboratory, exposure room, animal necropsy room, shower room, and three total-exhaust animal rooms. A barrier with plate glass windows separates the exposure control laboratory from the exposure room, with all monitor and control lines passing through sealed bulkheads affixed to the barrier wall. Double door pass-through windows connect the exposure room to the animal necropsy room, and the quarantine and pre-exposure animal holding rooms are separated from the exposure room by an air lock to prevent cross contamination between animal rooms, or direct contamination from the exposure room. The entire exposure facility is provided with preconditioned, filtered, single-pass air.

Whole body exposures are performed in 0.3 m^3 stainless-steel Rochester chambers, with four chambers used for exposures and two chambers used to house air control animals. The system design allows use of any number of the exposure chambers simultaneously, and chamber temperature is maintained by an electronically controlled cooling unit. A manually adjustable blower pushes air through a split stream arrangement which allows control of the air velocity past the fog oil generator while maintaining a constant air flow through the chamber. Orifice plates are used to monitor air flow and enhance stream mixing, and perforated baffle plates installed in the top pyramid of the chamber complete the mixing process and provide homogeneous chamber aerosol distributions. Each chamber is

exhausted through a coalescing filter and high efficiency particulate air (HEPA) filter. Check valves are present in the exhaust system to prevent back-flow.

Generation of the test atmospheres is accomplished by a vaporization/condensation method. An aerosol generator designed and developed at Oak Ridge National Laboratory (ORNL) was modified to meet the requirements of the exposure system. Fog oil is generated by injecting bulk oil under pressure (using nitrogen as a carrier gas to avoid combustion) onto a Vycor glass heating element. Premature condensation of the oil vapor/nitrogen mixture is minimized by heat tape wrapped around the generator tube. This mixture is injected into the precooled chamber air stream where the oil vapor instantly condenses to form the aerosol particles. Vapor concentrations are varied in the range of 25-634 mg oil/liter N_2 to provide chamber fog oil concentrations from 0.4 - 10 mg oil/liter air.

Aerosol concentration in the exposure chamber is continuously monitored using RAM-1 aerosol monitors (G.C.A. Corporation), each equipped with an aerosol diluter fabricated by project personnel. The sensors in these monitors produce a signal generated by near forward light scattering, the intensity of which is proportional to chamber aerosol concentrations. Calibration curves, comparing the signal with aerosol concentrations determined from filter samples of chamber atmosphere, are developed to allow real-time continuous concentration monitoring. Concentration readings from the RAM monitors are collected at 5 minute intervals by computer, and continuously recorded on strip chart recorders. Chamber temperature is also displayed, monitored, and recorded for later reference.

The exposure system is monitored and controlled from a remote station in the exposure control laboratory. Magnehelic gauges are used for monitoring the pressure drop across the coalescing filter, the chamber pressure with respect to room ambient pressure, and the pressure drop across the chamber flow monitoring orifice. Generator nitrogen flow is monitored and controlled with a mass flow controller. All parameters, including the liquid oil injection rate, are controlled by manual adjustment of potentiometer circuitry, which is compatible with the computer control and monitoring system.

The process control unit is fabricated and assembled by the Adaptive Data Acquisition and Control Corporation (ADAC), and uses the PROSYS I operating system with 256K Bytes of memory custom designed for industrial process control. Peripheral devices include printer, a terminal/monitor, a floppy disk drive data storage unit, and a video monitor. The computer is interfaced to monitor the following system functions: chamber temperature, Vycor temperature, heat tape temperature, chamber negative pressure, and aerosol concentrations for each chamber.

The safety of operating personnel, equipment protection, and integrity of test data were of major concern throughout the development of the aerosol exposure facility. Safety systems were incorporated to guard against laboratory exhaust system failure, chamber exhaust failure, and overheating of the generator. Photohelic gauges monitor room and chamber exhaust conditions, and pressure transducers monitor chamber pressure. Vycor heating elements are protected from overheating by a safety device in the primary controller. Because excessive temperature generated by the heating elements poses a serious safety threat, an independent temperature monitor is used as a secondary safety device. If system failures occur, such as loss of exhaust blower and/or chamber negative pressure, or over-temperature of the generator heating element, the safety system deactivates the generator heaters and closes a solenoid valve on the oil supply line. These system failures are signaled by audible and visual alarms. Once the safety system has responded to a system failure, the generation system will not restart until it is reset manually.

Filter sample mass collections are determined gravimetrically, and droplet size studies are performed periodically using a 7 stage cascade impactor. Mass loadings of each stage were also determined gravimetrically. In all tests performed with the impactor, over 90% of the mass was collected on stage 6 (cutpoint diameter of $0.65\ \mu\text{m}$) and stage 7 (cutpoint diameter of $0.43\ \mu\text{m}$). These data indicate that the diameter of the generated aerosol is essentially monodisperse, having an aerodynamic diameter in the range of 0.43 to $0.62\ \mu\text{m}$ ($\sigma_g \sim 1.5$).

A chemical analysis method has been developed to signal the occurrence of major chemical changes in the fog oil aerosol which result from changes in generation temperatures, oxidation of the oil during some phase of aerosol generation, or chemical variations in the liquid oil used for aerosol generation. If a major chemical change is observed, investigators can initiate appropriate corrective action.

High performance liquid chromatography (HPLC) effectively separates and quantitates the various compound classes found in the oil. HPLC coupled with UV detection provides a consistent profile of the unsaturated aliphatics, single and multiple ring aromatics, and semipolar (oxygen containing) compounds. Chemical shifts can be detected from the HPLC profile as changes in peak area (height) of the various compound class. Statistical analysis of the peaks given by the HPLC profile provides a reliable means to monitor chemical changes in the exposure atmospheres.

3. TOXICOLOGICAL TESTING

Toxicological testing of such complex mixtures is complicated, costly, and requires close collaboration among engineers, chemists, and biologists. The monitoring of the exposure atmospheres is difficult, especially when the chemicals react to form secondary by-products that may in themselves be of toxicological importance. Thus, any such toxicological study must be carefully designed and planned prior to initiating any health effects study.

This study was designed in three phases. The data collected after the completion of each phase will provide vital information that is necessary in making scientific decisions regarding the direction and design of future studies that look at the effects of long term exposure to this obscurant.

The purpose of this section of the manuscript is not to provide the reader with detailed medical data from these studies. These data will be presented at a future health effects symposium. Instead, the goals and objectives of this presentation are to provide the participants at this specialized meeting (Smoke/Obscurant Symposium VII) useful information on how one can evaluate the potential hazards posed by exposure to this smoke.

EXPERIMENTAL DESIGN

The first set of experiments conducted was performed to determine the level of inhalation exposure that results in an acute LC_{50} response in rats following a single 2, 3.5 or 6.0 hour exposure. The effects of such an exposure on histopathology in the nasal, tracheobronchial and alveolar regions will also be investigated. Acute toxicity studies are of scientific importance since they provide data to determine the median lethal concentration of a substance and permit estimation of the toxicity of this substance relative to that of other similar chemicals. Such studies could also provide data to approximate the chemical's mode(s) of action; to determine its specific toxic effect(s) on target organs and functions; and to provide a simulation of a variety of important occupational and environmental exposures including accidents and episodes of high concentrations. However, in this study the data provided an estimate of the toxicity of the fog oil, and was generated primarily to provide guidelines for the design of longer term studies.

Human exposure situations often involve repeated high concentration exposures, usually cyclic in nature, involving fractionated dose patterns. Utilizing the acute data base, the second phase (i.e. repeated exposures) has been initiated. These are range finding studies designed to examine a number

of different biological parameters after exposure for four weeks in a 3x2x2 experimental design. The three variables include the concentration of the fog oil (0, 0.5, and 1.5 mg/l); the frequency of exposure per week (2 or 4 times per week), and the duration of the exposure (1.1 or 3.5 hrs.). The data from these studies are being analyzed, and will be reported elsewhere at a later date when the experiments are completed. At the present time, an additional 4 week study at the same concentration levels, 4 days/week for 6 hour/day is being conducted.

The data generated from these experiments will also be utilized in designing the final phase of this study, which increases the exposure period to 90 days. By increasing the length of the exposure, there is a greater potential for the manifestation of disease. With longer exposures, the test chemical may deplete various host cellular defense mechanisms resulting in loss of cellular structure and function. The data collected from these investigations will provide a comprehensive study on the potential hazards posed by this obscurant so that health effects risks of such an exposure can be estimated.

EXAMINING FOR HEALTH EFFECTS

Several biological endpoints were selected to evaluate the host's response to this airborne mixture of test chemicals. These health indicators were carefully chosen to provide a battery of tests to evaluate both pulmonary and extra-pulmonary responses to the petroleum smoke.

In the initial acute toxicity studies, the observations recorded included the onset of any toxicological sign, survival time, lethality (LC_{50}), and gross pathology and histopathology. At this stage of the study, the effects were easily identifiable and obvious. With lower concentrations, the effects become more subtle, and the biological parameters measured must be more sensitive in order to detect small changes.

Since the respiratory system is the first target organ encountered by this chemical, a variety of techniques are available to measure pulmonary response to the inhaled fog oil obscurant. Tests must be selected to provide a broad array of information concerning changes in pulmonary function, biochemistry, morphology, and host defenses. Tests of small animal pulmonary function have now been developed in our laboratory and in the laboratories of other investigators. These tests are based on the same principles that are used in human pulmonary function testing, making the extrapolation of these animal data to human health effects more reliable. These tests can provide information that can often be directly related to a human adverse health effect. The pulmonary function tests that are available for use in this study are given in Table 1.

The ability of the lung to defend itself against airborne agents will be determined by evaluating the functioning of the pulmonary free cells (alveolar macrophages). These cells are involved in both the clearance of inhaled non-viable particles as well as in resistance to airborne infectious agents. The effect of the test chemical on these defense cells can be determined by examining the total number of free cells available. Differential counts of the pulmonary cells after exposure to the test substance will indicate if there is an influx of neutrophils and lymphocytes which tend to accumulate in the lungs during some types of injury. Such information is important in helping to understand the pathogenesis of chronic respiratory disease such as emphysema and fibrosis. Protein content of cell-free pulmonary lavage fluid will be measured as an indicator of serum protein (edema) in the pulmonary airways.

With prolonged exposure, the test chemicals or some reactive by-product may be transported to other tissues where they can cause significant health effects at sites distant from their point of entry. For this reason, one must also be aware of the buildup of these chemicals and the occurrence of significant extra-pulmonary health effects.

Clinical chemistry and hematology will be useful measurements to evaluate the potential target organ toxicity induced by fog oil. Blood counts, hematocrits, and hemoglobin levels will be included as routine hematology. In addition, approximately 20 different clinical chemistries will be run on serum from the exposed animals. For example, routine assays will be performed to measure albumin, alkaline phosphatase, bilirubin, SGOT, SGPT, BUN, glucose, etc.

Inhaled materials can also influence behavior in several ways. Studies in the laboratory have indicated that behavior modifications, seen in animals as well as humans, are complex. However, because of the major impact these behavioral effects might have on the human subject, behavioral studies with laboratory animals are becoming increasingly important, and are being included in more and more toxicological studies. In the fog oil study, animals will be tested for motor activity in a figure-eight maze. Such behavioral tests will assess motor and sensory function as indicators of possible damage to the nervous system.

Two other measurements being used in the fog oil study to detect extra-pulmonary effects include the effect of the test chemical on xenobiotic metabolism (sodium pentobarbital sleeping time and zoxazolamine paralysis time), and selected functional aspects of the immune system. Two types of immune functions will be examined, a) natural killer cell activity in spleen cells, and b) response of spleen and peripheral blood white cells to concanavalin A, phytohemagglutinin, and pokeweed mitogen.

Assays of potential immunotoxic effects are important and relevant because suppression of the immune response might be expected to enhance the host's susceptibility to infectious and neoplastic disease.

Thus, in these studies we have attempted to use a balanced battery of multiple endpoints. These endpoints are sensitive enough to detect adverse responses at not only various levels of the respiratory system, but also at other sites beyond the pulmonary system. Such a balanced study greatly increases the probability that subtle, important responses will not be inadvertently missed.

TABLE I. EXAMPLES OF PULMONARY FUNCTION TESTS IN SMALL ANIMALS

<u>Category</u>	<u>Tests</u>	<u>Physiological Interpretation</u>
Ventilatory Exchange	Respiratory rate Tidal Volume Minute Ventilation	Frequency of breathing Volume of breathing Total volume of air inspired or expired per minute.
Static Lung Volumes	Total lung capacity Vital capacity Residual volume	Elasticity of lungs Elasticity of lungs Elasticity of lungs
Respiratory Mechanics	Static Lung Compliance Dynamic Lung Compliance Airway Flow Resistance	Stiffness of respiratory system. Stiffness of respiratory system. Flow resistance of airways.
Distribution of Ventilation	Closing Volume Nitrogen washout	Closure of airways Distribution of ventilation
Diffusing Capacity	CO diffusion	Evaluate thickness of air-blood alveolar barrier
Pulmonary Edema	Wet/dry weight; Protein content in lavage fluid	Estimates extra-vascular fluid accumulation in the lung, edema.